## REMARKS

Courtesies extended to Applicant's representative, Sheila Kirschenbaum, in a telephone discussion with Examiner Goldberg on May 10, 2001, are acknowledged with appreciation.

Claims 10 to 24 are under consideration in the present application. By this communication, claim 12 has been canceled and claims 10 and 21 have been amended. No new matter is added by the amendments submitted herewith as all claim language is fully supported by the specification and originally filed claims. Attached herewith as Exhibit A is a marked-up version to show changes made to the specification and claims.

### Regarding the Specification

The disclosure is objected to because it contains embedded hyperlinks. In response to the objection, the specification has been amended to delete each occurrence of an embedded hyperlink and insert the respective uniform resource locator (url) in an information-only form, *i.e.*, not a hyperlink. Applicants respectfully submit that the amended specification is fully compliant with the guidelines in Manual of Patent Examining Procedure (MPEP) § 608.01.

The specification has also been amended to correct an inadvertent typographical error in the title of Table 5. As such, the amendment adds no new matter.

### Rejection Under 35 U.S.C. § 112

The rejection of claims 10 to 13, 19 and 22 to 24 under 35 U.S.C. § 112 as allegedly lacking enablement is respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion that the claims are broadly drawn to a method for detecting any cellular proliferative disorder by detecting the methylation state

of any nucleic acid. Amended claim 10 recites a method for detecting a cellular proliferative disorder associated with APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4 in a subject. The method includes contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of at least one gene or associated regulatory region of the gene. Only the methylation state of one or more specific genes including APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 and SDC4 is embraced by amended claim 10.

Applicants disagree with the Examiner's assertion that the specification does not provide "any correlation between tumor and normal tissue regarding hypermethylation of APOB, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 and SDC4 such that the skilled artisan would be able to take the information and detect cellular proliferative disorders", (Office Action , page 5, paragraph 2). Applicants direct the Examiner's attention to Table 5 at page 39 of the specification which provides a listing of genes that are differentially methylated in disease tissue in comparison with normal tissue. For example, hypermethylation of ABOB (apolipoprotein B) is associated with common tumors; hypermethylation of CDX2 (caudal type homeo box transcription factor 2) is associated with leukemias, breast cancer and prostate cancer; hypermethylation of EGFR (epidermal growth factor receptor) is associated with leukemias and breast cancer; and hypermethylation of FBN1 (fibrillin-1) is associated with leukemias, colon cancer, breast cancer and prostate cancer. Table 5 further provides that hypermethylation of GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 and SDC4 are associated with various cellular proliferative disorders. In addition, the region of the each gene that is hypermethylated, *i.e.* the regions of APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1and SDC4 containing CpG islands, is shown in Figure 4 and the nucleic acid sequences of each region are provided in SEQ

ID NO:105 and SEQ ID NO:7 to SEQ ID NO:119. With the guidance provided in the specification, one of skill in the art would readily be able to practice invention methods for detecting a cell proliferative disorder associated with APOB, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4.

Accordingly, Applicants respectfully submit that the specification fully enables claims 10 to 13, 19, and 22 to 24, and request reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

**Rejection Under 35 U.S.C. § 112, second paragraph**

The rejection of claims 10 to 24 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite is respectfully traversed.

Claims 10 to 24 are allegedly indefinite because of the recitation of "cellular proliferative" in claim 10 (at line 8) and because "cellular proliferative" allegedly lacks antecedent basis. Applicants submit that those of skill in the art would understand exactly what is intended by the term "cellular proliferative". However, in order to facilitate prosecution of the present application, claim 10 has been amended as suggested by the Examiner to recite "cellular proliferative disorder".

Claim 21 is allegedly indefinite because it is not clear which consecutive primer pairs are intended by the claim language. In response, claim 21 has been amended to recite each primer pair embraced by claim 21.

**Rejection Under 35 U.S.C. § 102(b)**

The rejection of claims 10 to 11, 13, 19 and 22 to 24 under 35 U.S.C. § 102(b) as allegedly being anticipated by Nelson *et al.* (U.S. Patent No. 5,552,277; hereinafter "Nelson") is respectfully traversed.

The present invention is directed to a method for detecting a cellular proliferative disorder associated with aberrant methylation of the gene APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4. The method to determine aberrant methylation includes contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of at least one of the genes or an associated regulatory region of the genes. Only Applicants provide methylated forms of the genes APOB, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4. Only Applicants identified the T-type calcium channel, CACNA1G, and its association with proliferative cell disorders such as colorectal cancers, colorectal adenomas, gastric cancers and acute myelogenous leukemia. In contrast, Nelson teaches a method of detecting a prostate cancer by determining the presence of hypermethylated glutathione-S-transferase (GSTP1). Nelson does not teach or suggest that any other cell proliferative disorders can be detected by invention methods, nor does Nelson teach that methylation of any genes other than GSTP1 are indicative of prostate cancer or any other cell proliferative disorder. Therefore, Nelson does not anticipate Applicants' invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 10 to 11, 13, 19 and 22 to 24 under 35 U.S.C. § 102(b).

The rejection of claims 10 to 11, 13, 19 and 22 to 24 under 35 U.S. C. § 102(b) as allegedly being anticipated by Baylin *et al.* (U.S. Patent No. 5,756,668, hereinafter "Baylin") is respectfully traversed.

Applicants invention relates to a method for detecting a cellular proliferative disorder associated with the gene APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4 by detecting aberrant methylation of the genes. In contrast, Baylin teaches that one gene, a tumor suppressor gene named hypermethylation in cancer 1 (HIC-1), is aberrantly hypermethylated in multiple common tumor types. Baylin does not teach or suggest that hypermethylation of any specific gene other than HIC-1 is associated with tumors. Therefore, Baylin does not anticipate Applicants' invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 10, 11, 13, 19 and 22 to 24 under 35 U.S.C. § 102(b).

**Double Patenting Rejection**

The rejection of claims 10, 11, 13 and 22 to 24 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1 and 3 of U.S. Patent No. 5,552,227 ("Nelson") is respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion that the claims of the present application encompass the claims of Nelson. Amended claim 10 and claims dependent therefrom relate to a method for detecting a cellular proliferative disorder associated with the gene APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4. The method includes contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of at least

one gene or associated regulatory region of the gene including APOB, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 and SDC4. Claims 1 and 3 of Nelson relate to a method of detecting a prostatic cell proliferative disorder associated with glutathione-S-transferase. Nelson does not teach or suggest any cell proliferative disorder other than prostatic cell proliferative disorder. Furthermore, Nelson does not teach or suggest determining the methylation state of APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1 or SDC4. Therefore, Applicants submit that the present claims are patentably distinct from claims 1 and 3 of Nelson. Accordingly, reconsideration and withdrawal of the double patenting rejection of claims 10, 11, 13 and 22 to 24 are respectfully requested.

PATENT
Attorney Docket No.: JHU1590

In view of the above amendments and remarks, reconsideration and favorable action on all pending claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is requested to telephone Applicant's representative, Lisa A. Haile, J.D., Ph.D., at (858) 677-1456, so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: May 15, 2001

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Attachment: Exhibit A

PATENT

In re Application of:
Jean-Pierra Issa
Application No.: 09/398,522
Filed: September 15, 1999
Exhibit A: Page 1

Attorney Docket No.: JHU1590

**SPECIFICATION AND CLAIMS MARKED-UP TO SHOW CHANGES MADE**

**In the Specification:**

At page 11, lines 1-15:

Methylated nucleic acid sequences are also provided. For the first time, the present invention provides methylated chemical structures for the following genes: APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1, and SDC4. One of skill in the art can now readily locate the CpG-rich sequences associated with these genes and identify such methylated forms of the genes/regulatory sequences by methods described herein (The gene sequences can be identified in a gene database found at [http://www.ncbi.nim.nih.gov/UniGene/index.html] the following uniform resource locator (url): ncbi.nim.nih.gov/UniGene/index.html). The invention provides CpG-rich regions from the above genes as set forth in SEQ ID Nos:105-119. Thus, in yet another embodiment, the invention provides an isolated nucleic acid molecule having at least one methylated Cytosine of a CpG dinucleotide in a CpG-rich region and encoding a gene selected from APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1, and SDC4. The methylated C residue of a CpG dinucleotide is located within a CpG-rich region selected from SEQ ID NO:105-118 and SEQ ID NO:119.

PATENT

In re Application of:
Jean-Pierre Issa
Application No.: 09/398,522
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Exhibit A: Page 2

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At page 35, line 40, please delete, "http://www.ncbi.nim.nih.gov/UniGene/index.html", and substitute therefor, –the following url address: ncbi.nim.nih.gov/UniGene/index.html–.

Note: Y = C or T, R = G or A; row 1-SEQ ID NO:1 and 2; row 2-SEQ ID NO:3 and 4 and so forth through to SEQ ID NO:32, respectively. The gene sequences can be found in a gene database found at [http://www.ncbi.nim.nih.gov/UniGene/index.html] the following uri address: ncbi.nim.nih.gov/UniGene/index.html.

At page 67, lines 13-25:

Six g of total RNA, was reverse transcribed using the SUPERSCRIPT kit (GIBCO-BRL) for first strand cDNA synthesis. One hundred ng of cDNA was used as template for RT-PCR reactions. To design the RT-PCR primers, Blast search was performed using the rat CacnalG cDNA sequence (Genbank AF027984) reported previously (25) and exon-intron boundaries of the human CACNA1G were predicted by this analysis. Each primer set was designed to amplify the cDNA across several introns. Primer sequences and PCR conditions are available, at [http://www.med.jhu.edu/methylation/primers] the following uniform resource locator (url): med.jhu.edu/methylation/primers. GAPDH was also amplified as a control using primers GAPDHF: 5'-CGGAGTCAACGGATTGGTCGTAT-3' (SEQ ID NO:55) and GAPDHR: 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:56). All reactions were performed with RT(-) controls. PCR amplification was performed for 35 cycles of 95° C 30 sec, 60-65°C for 30 sec, 72°C for 30 sec, and the products were analyzed by agarose gel electrophoresis.

At page 68, lines 1-17:

PCR reaction products were precipitated with ethanol, resuspended in diluted water and cloned into the pCR2.1 vector using the TA cloning kit (Invitrogen) according to the manufacturer's instruction. After transformation, plasmid DNA was purified using the Wizard Miniprep Kit (Promega). DNA sequence analysis was carried out at the Johns Hopkins University Sequence Facility using automated DNA sequencers (Applied Biosystems). Sequence homology was identified by the BLAST program of the National Center for Biological Information (NCBI) available at [http://www.ncbi.nlm.nih.gov/BLAST] the following uniform resource locator (url): ncbi.nlm.nih.gov/BLAST/. An IMAGE cDNA clone (Genbank: H13333) was identified by BLAST analysis using the sequence of BAC AC004590 (Genbank) which includes MINT31. Putative genes (G1 and G2) were identified by GENSCAN (available at [http://ccr-081.mit.edu/GENSCANMIT.htm1] the following uniform resource locator (url): ccr-081.mit.edu/GENSCANMIT.htm1) using the BAC sequence data. IMAGE cDNA clone H1333) was then obtained from the American Type Culture Collection and completely sequenced. Potential transcription factor binding sites and promoter prediction were examined using the TESS and TSSG programs respectively, available at the Baylor College of Medicine BCM Launcher ([http://kiwi.imgen.bcm.tmc.edu:8088/search launcher/launcher.html] the following uniform resource locator (url): kiwi.imgen.bcm.tmc.edu:8088/searchlauncher/launcher.html/). The nucleotide sequence of part of the 5' end of the cDNA of CACNA1G has been submitted to Genbank.

PATENT

In re Application of:
Jean-Pierre Issa
Application No.: 09/398,522
Filed: September 15, 1999
Exhibit A: Page 4

Attorney Docket No.: JHU1590

At page 39, in the title of Table 5:

Table 5 New genes differentially methylated in disease versus normal [issue] tissue

**In the Claims:**

10. (Amended) A method for detecting a cellular proliferative disorder associated with APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1or SDC4 in a subject comprising:

a) contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of at least one gene or associated regulatory region of the gene;

wherein the gene is selected from the group consisting of APOB, CACNA1G, CDX2, EGFR, FBN1, GPR37, HSPA6, IQGAP2, KL, PAR2, PITX2, PTCH, SDC1, SDC4 and combinations thereof and

b) identifying aberrant methylation of regions of the gene or regulatory region, wherein aberrant methylation is identified as being different when compared to the same regions of the gene or associated regulatory region in a subject not having said cellular proliferative disorder, thereby detecting a cellular proliferative disorder in the subject.

21. (Amended) The method of claim 20, wherein the [primers are in consecutive pairs] primer pair is selected from the group consisting of [SEQ ID NO:1-49 and SEQ ID NO:50] SEQ ID NO:1 and 2, SEQ ID NO:3 and 4, SEQ ID NO:5 and 6, SEQ ID NO:7 and 8, SEQ ID NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:13 and 14, SEQ ID NO:15 and 16, SEQ ID NO:17 and 18, SEQ ID NO:19 and 20, SEQ ID NO:21 and 22, SEQ ID NO:23 and 24, SEQ ID NO:25 and 26, SEQ ID NO:27 and 28, SEQ ID NO:29 and 30, SEQ ID NO:31 and 32, SEQ ID NO:33 and 34, SEQ ID NO:35 and 36, SEQ ID NO:37 and 38, SEQ ID NO:39 and 40, SEQ ID NO:41 and 42, SEQ ID NO:43 and 44, SEQ ID NO:45 and 46, SEQ ID NO:47 and 48, and SEQ ID NO:49 and 50.

21. (Amended) The method of claim 20, wherein the [primers are in consecutive pairs] primer pair is selected from the group consisting of [SEQ ID NO:1-49 and SEQ ID NO:50] SEQ ID NO:1 and 2, SEQ ID NO:3 and 4, SEQ ID NO:5 and 6, SEQ ID NO:7 and 8, SEQ ID NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:13 and 14, SEQ ID NO:15 and 16, SEQ ID NO:17 and 18, SEQ ID NO:19 and 20, SEQ ID NO:21 and 22, SEQ ID NO:23 and 24, SEQ ID NO:25 and 26, SEQ ID NO:27 and 28, SEQ ID NO:29 and 30, SEQ ID NO:31 and 32, SEQ ID NO:33 and 34, SEQ ID NO:35 and 36, SEQ ID NO:37 and 38, SEQ ID NO:39 and 40, SEQ ID NO:41 and 42, SEQ ID NO:43 and 44, SEQ ID NO:45 and 46, SEQ ID NO:47 and 48, and SEQ ID NO:49 and 50.